



MacConkey Agar

Selective and differential medium for detection of Enterobacteriaceae from clinical samples and other materials.

INTENDED PURPOSE

Slightly selective medium for the differentiation between lactose-fermenting and lactose-non-fermenting Gram-negative enteric bacilli from faeces, urine, foodstuffs, wastewater and other materials of sanitary importance. This medium is intended as an aid in the diagnosis, requiring additional tests to complete the diagnostic results.

DESCRIPTION

MacConkey Agar is a slightly selective and differentiating agar that only grows gram-negative bacterial species; it can further differentiate the gram-negative organisms based on their lactose metabolism. The fermentation of lactose produces organic acids, particularly lactic acid, which decreases the pH of the agar. This medium contains a pH indicator that turns pink under acidic conditions. Therefore, lactose-fermenting-gram-negatives (lactose-fermenters) will form pink colonies, while non-lactose fermenters will form off-white opaque colonies. Even within lactose-fermenters, species will show a varying rate of growth. This medium is prepared according to recommendations of the harmonized USP/EP/JP method for the detection of *E. coli* in non-sterile pharmaceutical products.

TYPICAL FORMULA*

(g/litre)

| | |
|------------------------------|--------|
| Pancreatic Digest of Gelatin | 17.0 |
| Peptone from Meat | 1.5 |
| Peptone from Casein | 1.5 |
| Lactose | 10.0 |
| Sodium Chloride | 5.0 |
| Bile Salts | 1.5 |
| Agar | 15.0** |
| Neutral Red | 0.03 |
| Crystal Violet | 0.001 |
| Final pH 7.1 ± 0.2 at 25°C | |

*Adjusted and/or supplemented as required to meet performance specifications.

** Adjusted according to gel strength to meet performance specifications.

METHOD PRINCIPLE

Pancreatic digest of gelatin and peptone from meat and casein provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Lactose is the fermentable carbohydrate. Sodium chloride maintains the osmotic balance of the medium. Bile salts and crystal violet are the selective agents, inhibiting Gram-positive organisms and allowing Gram-negative bacteria to grow. Agar is the solidifying agent. Neutral red is the pH indicator.

PREPARATION

Dehydrated medium

Suspend 51.5 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil for 1 minute shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes.

Medium in bottles

Melt the content of the bottle in a water bath at 100°C (losing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as: Autoclave, water bath, sterile Petri plates, test tubes, inoculating loops, swabs, incubator, quality control organisms.

SPECIMENS

Clinical specimens should be sampled at the acute stage, before antimicrobial therapy (where possible) and examined as soon as possible after collection.

Sample can be inoculated directly onto the agar. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied. Refer to specific guidelines for more information about specimen collection and preparation.

TEST PROCEDURE

Ensure there is no visible moisture on the plates before use.

Inoculate the plates by directly streaking the specimen on the agar surface or spread the sample from an enrichment culture. Incubate aerobically at $35 \pm 2^\circ\text{C}$ for 18-24 h.

To isolate *E. coli* in pharmaceutical products, the Harmonized USP/EP/JP method recommends carrying out a two steps enrichment procedure by inoculating the sample in Tryptic Soy Broth and afterwards in MacConkey Broth. Subculture on a plate of MacConkey Agar and incubate aerobically at $30\text{-}35^\circ\text{C}$ for 18-72 hours.

INTERPRETING RESULTS

Lactose-non fermenting organisms, such as *Salmonella*, *Shigella* and *Proteus* spp., form colorless or clear colonies.

Lactose-fermenting organisms, such as *E. coli* and *Klebsiella* spp., grow as pink to red colonies with or without a zone of precipitated bile.

Enterococci, staphylococci and other Gram-positive bacteria are partially or completely inhibited.

STORAGE

The powder is very hygroscopic, store the powder at $10\text{-}30^\circ\text{C}$, in a dry environment, in its original container tightly closed. Store bottles and prepared plates at $10\text{-}25^\circ\text{C}$ away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.

Medium in bottles: 2 years.

Ready-to-use plates: 6 months.

QUALITY CONTROL

Appearance of Dehydrated Medium: free-flowing, homogeneous, beige-pink.

Appearance of Prepared Medium: slightly opalescent, pinkish red.

Expected Cultural Response:

| Control strain | Inoculum | Incubation | Criteria | Specification | |
|-------------------------------|-------------|---|---------------------------------------|-----------------------------------|--|
| <i>Salmonella</i> Typhimurium | ATCC® 14028 | 50-100 CFU | 18-24 h / $35 \pm 2^\circ\text{C}$ | Good growth ($P_R \geq 0.5$) | Colourless colonies |
| <i>Shigella flexneri</i> | ATCC® 12022 | | | | Colourless colonies |
| <i>Proteus mirabilis</i> | ATCC® 12453 | | | | Colourless colonies |
| <i>Klebsiella pneumoniae</i> | ATCC® 13883 | | | | Pink colonies |
| <i>Escherichia coli</i> | ATCC® 8739 | 18-72 h / $32.5 \pm 2.5^\circ\text{C}$ | | | Pink colonies with a zone of precipitated bile |
| <i>Enterococcus faecalis</i> | ATCC® 29212 | $10^4\text{-}10^6$ CFU | 18-24 h / $35 \pm 2^\circ\text{C}$ | - | Partial to complete inhibition, very small opaque colonies |
| <i>Staphylococcus aureus</i> | ATCC® 25923 | | | | - |

A productivity ratio (P_R) of 0.5 is equivalent to a recovery rate of 50%.

Please refer to the actual batch related Certificate of Analysis (CoA).

PERFORMANCE CHARACTERISTICS

Performance testing of MacConkey Agar was carried out using the QC strains listed above. The results obtained met the established criteria.

LIMITATIONS

Invalid results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology. The operator should understand the principles of the procedures, including its performance limitations, in advance of operation to avoid potential mistakes.

Growth depends on the requirements of each individual organism. It is therefore possible that certain strains which have specific requirements (substrate, temperature, incubation conditions, etc.) may not develop.

MacConkey Agar is intended as an aid in the diagnosis of infectious diseases, requiring further tests to complete the diagnostic results.

WARNING AND PRECAUTIONS

- 1) **For *in vitro* diagnostic use (IVD).**
- 2) **For laboratory professional use only.**
- 3) Operators must be trained and have certain experience. Please read the instructions carefully before using the product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.
- 4) Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.
- 5) Do not use if the product or packaging appears to be damaged.
- 6) Follow standard precautions. All patient specimens should be considered potentially infectious and handled accordingly.
- 7) Handle all specimens as if infectious using safe laboratory procedures. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
- 8) Avoid cross-contamination of samples by using disposable tips and changing them after each sample.
- 9) Do not mix reagents of different batches. Please use the product within the validity period.
- 10) Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- 11) Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors.
- 12) Ensure laboratory equipment is calibrated and maintained in accordance with the laboratory's procedure.
- 13) When test results are transmitted from the laboratory to an informatics centre, attention has to be done to avoid erroneous data transfer.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

The product is available in the various configurations listed below. There may be additional product ref. numbers as well. For an updated listing of available products, visit liofilchem.com

| Product | Format | Packaging | Ref. |
|----------------|------------------|------------|---------|
| MacConkey Agar | Plate 90 mm | 20 plates | 10029 |
| | | 100 plates | 10029* |
| | Bottle | 6 x 100 ml | 402240 |
| | | 6 x 200 ml | 412240 |
| | | 6 x 500 ml | 470090 |
| | Dehydrated media | 500 g | 610028 |
| | | 100 g | 620028 |
| | | 5 kg | 6100285 |

Revision History

| Revision | Release Date | Change Summary |
|----------|--------------|--|
| 0 | 2024-03-12 | Updated layout and content in compliance with IVDR 2017/746, version reset to revision 0 |

In case of malfunctions or defects, contact immediately Liofilchem (*) or the local representative.

In case of incident associated with the device, notify immediately Liofilchem (*) or its local representative and the National Competent Authority.

*Please login to <https://www.liofilchemstore.it/login.php> (user ID and password required) and click on Complaint.

This IFU document and the SDS are available from the online Support Center:

[liofilchem.com/ifu-sds](https://www.liofilchem.com/ifu-sds)